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Microwave use brings significant advantages to histoprocessing of orthopaedic tissues

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Purpose: Histology plays a critical role in cartilage repair research. However, routine manual histoprocessing for orthopaedic tissues remains laborious, time-consuming and requires toxic chemicals. The current study investigated the ability of microwave technology (Milestone) to process osteochondral and synovial samples, and compared histological quality and reproducibility to standard manual histoprocessing.
Methods and Materials: 8 rabbit femoral articular cartilage and patellar synovial tissue samples, and 3 human osteochondral samples were decalcified, trimmed, and each half subjected to either standard manual or microwave histoprocessing in paraffin. Embedded osteochondral samples were sectioned to 5 µm and either stained with Safranin-O/Fast Green or immunostained for collagen type II. Synovial sections were stained with H&E.
Results: Microwave processing substantially decreased processing time from 46 to 3.25 hours for cartilage, and 8 to 3.25 hours for synovial tissue, without the use of toxic xylene and toluene. Both methods produced high quality tissue sections that underwent minimal shrinkage, contained very few artifacts, and did not exhibit swelling of connective tissue fibers. Cartilage zones contained chondrocytes with good morphological characteristics and the subchondral bone contained osteoclasts with few morphological alterations. Cartilage GAG staining intensity observed with Safranin-O was reproducible for both histoprocessing methods, as was collagen type II staining. However, only microwave processing preserved bone marrow structures and allowed differentiation between marrow cell types.
Conclusions: Microwave histoprocessing consistently produced high quality, reproducible and equivalent histological results when compared with routine manual histoprocessing for both osteochondral and synovial tissue samples. Microwave use, however, demonstrated significant benefit through much shorter histoprocessing times in a safer environment.

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Effect of Intraarticular Growth Factor Injections on Cartilage Repair in a Rat Model of Acute Chondral Injury

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Purpose: To evaluate the effect of intraarticular injections of growth hormone and insulin-like growth factor-1 on cartilage repair in a rat model of partial thickness cartilage injury.
Methods and Materials: Thirty-five Sprague-Dawley rats were used in a model of partial-thickness cartilage injury to assess the effect of intraarticular growth factors on cartilage repair. A micro-curette was used to create a cartilage defect on each side of the trochlear groove in both knees of each animal. One week later, intraarticular injections were begun and continued bi-weekly for 3 weeks, totaling 6 injections in each knee. In all animals, the left knee served as a control and was injected with 0.1mL sterile saline. The right knee received treatment and animals were randomly assigned to three groups. Group 1 (N=12) was injected with 0.1mL sterile saline, group 2 (N=12) with 0.1mL purified rat growth hormone (Prospec Labs, 250µg/ml) and group 3 (N=11) with 0.1mL purified rat IGF-1 (Prospec Labs, 250µg/ml). All rats were sacrificed six weeks after the injections were completed and the distal femurs were harvested and prepared for histology (H&E, safranin-O/fast green and Alcian Blue. Cartilage was graded based on the appearance of the surface, matrix, cell distribution and subchondral bone.
Results: In group 1 (saline) there was no significant difference in cartilage scores between the right and left knees. In addition, there was no difference between scores for left knees (saline) in groups 2 and 3 and the scores for group 1. Average cartilage scores for the treatment groups are summarized in table 1. IGF-1 had significantly higher scores than the saline control for surface, matrix and cell distribution. There was no statistically significant difference between GH and control or GH and IGF-1 for any cartilage score.
Conclusions: There is currently no effective treatment for partial-thickness cartilage injuries. This study demonstrates that insulin-like growth factor-1, when injected into the joint, enhances cartilage repair after acute injury. Growth hormone showed a trend towards producing higher cartilage scores compared with a saline control but this was not statistically significant. Intraarticular growth factors may have a role in enhancing cartilage repair after partial-thickness chondral injury.

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Administration of TGF beta-1 during the expansion of human articular chondrocytes may trigger the ontogenesis of endochondral bone formation in the cultured cells

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Purpose: Cell-based cartilage resurfacing (ACI) requires the ex-vivo expansion of autologous articular chondrocytes. Defined culture conditions have been devised to minimise phenotypic changes consequent to the loss of a 3D environment. However these conditions must be carefully assessed to avoid reactivating the ontogenesis of endochondral bone formation in dedifferentiated articular cells. Interestingly, in proliferating cultured chondrocytes, TGFß-1 regulates the expression of cartilage proteins but also contributes to hindering the differentiation potential of those ex-vivo expanded cells. We therefore evaluated if TGF administration could unlock the immature articular stage and reactivate the endochondral ossification fate in cultured human articular cells.
Methods and Materials: To this purpose human primary articular chondrocytes were expanded in a serum-free medium (SF), with or without TGFß-1 (TGF). Cell aliquots were maintained in alginate or as micromasses for immunocitochemistry and TUNEL analysis, or replated in osteogenic medium. RT-PCR and mineralization assays were performed after the expansion phases and the osteogenic inductions, for both amplification conditions.
Results: In chondrogenic 3D culture systems, TGF-expanded cells showed a partial loss of matrix components, as assessed by Alcian blue, anti-aggrecan and anti- type II collagen immunocitostainings. Positivity was evidenced for RAGE, IHH, type X collagen, and for the presence of apoptotic cells, paralleling a reduction of BCL-2 levels. After osteogenic induction, TGF-expanded cells strongly mineralised, displaying an increased osteocalcin level.
Conclusions: Thus exposure to TGFß-1 triggers the onset of the endochondral maturation pathway, possibly in dedifferentiated cells still able to undertake either the articular or the endochondral differentiation. The latter fate is clearly detrimental to ACI attempts.

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Subperiosteal injection of growth factors improves in vitro periosteal cartilage formation

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Purpose: To determine the potential of subperiosteal injection of TGF-b1 and IGF-I, alone or in combination, to enhance in vitro periosteal cartilage formation and overcome the age-related decline in chondrogenic potential
Methods and Materials: 111 male New Zealand white rabbits (6 months, 1 year, or 2 years old) were anesthetized, injected (subperiosteally) with TGF-b1 (20 or 200ng), IGF-I (0.2 or 2 mg), TGF-b1 plus IGF-I (200 ng + 2 mg), or vehicle in the medial proximal tibia. Rabbits were sacrificed after 1, 3, 5 or 7 days. Periosteal explants were harvested from the injection sites and cultured for 6 weeks with DMEM 10% FBS in agarose suspension with 10 ng/mL TGF-b1 for the first 2 days. The explants were weighed, embedded in paraffin, sectioned and stained with Safranin O/fast green. Cartilage yield (% area) and total cartilage (mg) were determined by histomorphometry. Results were analyzed using 1, 2, or 3-factor ANOVA and means comparisons where appropriate.
Results: Injection of 200 ng TGF-b1 or the combined treatment of TGF-b1 plus IGF-I significantly increased cartilage production in all age groups, but not at all time points ($p<0.05$). IGF-I alone did not significantly enhance cartilage production in any group. However, a synergistic effect of TGF-b1 plus IGF-I was observed in the 2 year-old rabbits in the combined treatment group (>2-fold increase over TGF-b1 only).
Conclusions: These studies strongly suggest that it is possible to enhance the chondrogenic potential of periosteum by a one-time injection of growth factors, specifically TGF-b1 and TGF-b1 plus IGF-I.